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Transformational Principles for NEON Sampling of Mammalian Parasites and Pathogens: A Response to Springer and Colleagues

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Seen as an opportunity to establish a nationwide web of environmental monitoring sites (Kao et al. 2012), the National Environmental Observatory Network (NEON) is now releasing a series of protocols presented with apparently broad community support. Springer and colleagues (2016) outlined sampling designs aimed at understanding how changing environmental conditions will affect mammals and associated parasites. They present a compelling case:

> [Parasites] can be important drivers of ecological and evolutionary changes in natural, agricultural, and urban ecosystems. . . . These changes could also have unanticipated effects on ecological communities at large, particularly when individuals of constituent species play influential roles in community-level interactions or ecosystem function.
>
> . . . As the size and scope of surveillance efforts expand, appropriate sampling design and methodological standardization will greatly facilitate comparisons across data sets and scales. Although logistically challenging, such large-scale, standardized sampling efforts are critical to characterize regional, continental, and multi-decadal patterns of disease dynamics. Insights gleaned from such projects hold promise for informing efforts to promote human health and wildlife conservation while furthering our fundamental understanding of the ecology and evolution of host–parasite interactions in natural systems.

Although the argument for studying parasites is clear, the proposed protocols are too limited to advance the understanding of ecological (much less evolutionary) responses to species invasions, changes in climate, and land use (Kao et al. 2012) or even the long-term dynamics of rodent–parasite systems. We offer a critique and an expanded vision for NEON's mammal–parasite protocols beyond select tick-, mosquito-, and rodent-borne pathogens. We encourage expanded sampling of (a) the parasite assemblage and (b) the host assemblage, along with (c) a two-pronged sampling design based on both rigorous sample archival and "mark-release" approaches. To fail to do either (a) or (b) excludes the possibility of both community- and ecosystem-level perspectives, and failure to do (c) effectively excludes sample-based studies and future work using yet-to-be-developed technologies. Reflecting on the long and highly productive history of specimen-based research (e.g., Suarez and Tsutsui 2004), we encourage NEON to take a more holistic, synergistic, and sample-intensive approach as a basis for long-term environmental observatories. Investment in NEON comes at a time when discoveries in ecology have exploded as a result of informatic and molecular tools that harness links among data types (e.g., geospatial, anatomical, genomic, physiological, and biological interaction traits). Tackling exceedingly complex environmental questions calls for holistic approaches that are reproducible and based on object-based links. Importantly, simple modifications would require minimal changes and costs to the expensive mark-release NEON protocols proposed and result in a much more productive platform.

To assess change, a broadly comparable infrastructure that includes deep sample archives across NEON sites is required. Sample-based research is huge and growing, with links among elements of biodiversity forming the crux for progress in both established and emerging fields. Microbiome, isotopic, and molecular ecology, as well as specimen-based metagenomics, transcriptomics, and proteomics, all depend on samples that can be tied back to the original organism. In addition, individual-level links represent the key for investigating networks of biotic interactions in community ecology and molecular biology (e.g., pathogen X found in tissue Y of mammal individual Z, corresponding to mammalian DNA sequence A and expression profile B; Morales-Castilla

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et al. 2015). Such sampling also reinforces the fundamentals of scientific research: verification, replication, extension, and integration across studies. To maximize impact, the infrastructure deployed in NEON should provide not only observational data but also comprehensive sampling of parasitic organisms and their mammalian hosts, along with a commitment to the permanent archiving of samples in appropriately accredited repositories.

As Springer and colleagues (2016) recognize, parasitism is a major driver of ecological systems (estimated at greater than 50 percent of all biodiversity) and represents an important source of zoonotic infections (e.g., Daszak et al. 2000, Hoberg et al. 2015), but the proposed NEON protocols lack a balanced effort to study parasites. For example, helminths are a highly diverse assemblage (three phyla parasitize rodents) that is best assessed by direct field sampling and archival development. We recommend separate removal traplines followed by comprehensive necropsies during specimen preparation. Because parasitic worms often have complex life cycles that are affected by numerous biotic and abiotic factors, they are exquisite indicators of environmental change. NEON's limited effort to build archives represents a missed opportunity to maximize the impact of federal funds over time. The irony of focusing entirely on mark-release efforts is that such restricted (and expensive) activities severely limit our view of known parasites and seldom result in discoveries of new pathogens. In a textbook example of an emerging infectious disease in modern times, museum archives led to the original discovery of the devastating Sin Nombre hantavirus (Yates et al. 2002, DiEuliis et al. 2016) and subsequent transformative revelations that hantaviruses have a deep history with mammals and are widespread not only in rodents but also in shrews, moles, and bats on multiple continents (Yanagihara et al. 2014).

More generally, limited sampling of diversity is unlikely to stimulate powerful integration among projects or to foster synthetic perspectives in ecology, evolution, or biogeography. NEON protocols (e.g., Springer et al. 2016) should engage the broader ecological community and take advantage of existing and proven biodiversity and ecological infrastructure (permanent museum archives with taxonomic expertise, Web-accessible and interconnected databases, object tracking of samples, digital links to publications and other research products, etc.). Rather than leverage existing assets and expertise, the proposed protocols attempt to reinvent a wheel that has already been fine tuned by decades of effort expended by countless experts in field sampling, specimen curation and loaning, and big-data management and connectivity.

Because NEON has suffered setbacks (Mervis 2015) and is still in the relatively early stages of implementation, now is the time to improve and enhance protocols for the next 30 years. Careful consideration should be given to the consequences of failing to develop temporally deep and spatially extensive archives of organisms. Available technology and the questions of interest will evolve in the coming decades—vouchering specimens from a broader taxonomic range will maximize opportunities for future insights and productivity. Protocols should be reconfigured to ensure the expanded development of extensive, holistic archives of diverse taxa with targeted sampling minimally during four periods annually at NEON sites. Each holistic specimen becomes an "observatory" of environmental conditions, so extensive archives for host species (multiple tissues) and associated parasites (helminths, arthropods, gut microbiomes, viruses, protozoa, and bacteria) will further facilitate access to basic ecological information and deep understanding about fine-scale interactions in complex host–parasite assemblages. Investment in this extended infrastructure is relatively inexpensive, and the value of such an approach is proven (Malmstrom et al. 2007, Moritz et al. 2008). In particular, integrated understanding of the linkages and dynamics of hosts and parasites is essential to providing a pathway to identify emerging impacts of environmental perturbation, direct threats to ecosystem integrity, and animal and human health (Brooks et al. 2014). A holistic foundation for NEON represents a singular opportunity to develop crucial, long-term infrastructure for North America and a powerful synergistic model for ecosystem assessment globally. Only through such approaches can the observatory truly transform our understanding of environmental change.

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